REMARKS

Entry of the above amendment prior to examination is respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims. The attached pages are captioned **"Version with Markings to Show Changes Made."**

I. Amendments

The specification has been amended in accordance with 37 C.F.R. §1.821 through 1.825 to add the Sequence Listing.

The specification and claims have been amended in accordance with 37 C.F.R. §1.821(d) to add sequence identifiers preceded by SEQ ID NO:.

No new matter is introduced by way of these amendments.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 846-7500.

Respectfully submitted,

Date: 4-3-03

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Version with Markings to Show Changes Made

In the specification:

On page 2, please replace the paragraph starting on line 25 with the following:

Ooi et al., *Curr. Genet.*, Vol. 18, pp. 217-222 (1990) disclose the cDNA sequence coding for endoglucanase F1-CMC produced by *Aspergillus aculeatus* which contains the amino acid strings NNLWG (SEQ ID NO: 1), ELMIW (SEQ ID NO: 66) and GTEPFT (SEQ ID NO: 3). Sakamoto et al., *Curr. Genet.*, Vol. 27, pp. 435-439 (1995) discloses the cCNA sequence encoding the endoglucanase CMCase-1 From *Aspergillus kawachii* IFO 4308 which contains the amino acid strings ELMIW (SEQ ID NO: 66) and GTEPFT (SEQ ID NO: 3). Ward et al., discloses the sequence of EGIII having the amino acid strings NNLWG (SEQ ID NO: 1), ELMIW (SEQ ID NO: 66) and GTEPFT (SEQ ID NO: 3). Additionally, two cellulase sequences, one from *Erwinia carotovara* and *Rhodothermus marinus* are disclosed in Saarilahti et al., *Gene*, Vol. 90, pp. 9-14 (1990) and Hreggvidsson et al., *Appl. Environ. Microb.*, Vol. 62, No. 8, pp. 3047-3049 (1996) which contain the amino acid string ELMIW (SEQ ID NO: 66). However, none of these references discloses or suggests that these amino acid strings have any particular relevance in identifying or isolating other cellulases, and particularly fail to suggest that such cellulases are obtainable from such diverse organisms as bacteria, Actinomycetes and other filamentous fungi.

On page 4, please replace the paragraph starting on line 1 with the following:

According to the present invention, an enzyme having cellulolytic activity is provided comprising an amino acid sequence comprising therein an amino acid string selected from the group consisting of:

(a) Asn-Asn-(Leu/Phe/Lys/Ile)-Trp-Gly (SEQ ID NO: 1)

(b) Glu-(Leu/Phe/Ile)-Met-Ile-Trp (SEQ ID NO: 2)

(c) Gly-Thr-Glu-Pro-Phe-Thr (SEQ ID NO: 3);

(d) (Ser/Tyr/Cys/Trp/Thr/Asn/Lys/Arg)-(Val/Pro)-(Lys/Ala)-(Ser/Ala)-(Tyr/Phe) (SEQ ID NO: 42);

(e) Lys-Asn-Phe-Phe-Asn-Tyr (SEQ ID NO: 5).

On page 4, please replace the paragraph starting on line 17 with the following:

In yet another embodiment of the present invention, a method of detecting an EGIII-like enzyme is provided comprising the steps of:

(1) preparing a DNA primer which encodes an amino acid string selected from the group consisting of:

(a) Asn-Asn-(Leu/Phe/Lys/Ile)-Trp-Gly (SEQ ID NO: 1)

(b) Glu-(Leu/Phe/Ile)-Met-Ile-Trp (SEQ ID NO: 2)

(c) Gly-Thr-Glu-Pro-Phe-Thr (SEQ ID NO: 3);

(d) (Ser/Tyr/Cys/Trp/Thr/Asn/Lys/Arg)-(Val/Pro)-(Lys/Ala)-(Ser/Ala)-(Tyr/Phe) (SEQ ID NO: 42); and

(e) Lys-Asn-Phe-Phe-Asn-Tyr (SEQ ID NO: 5).

(2) preparing genomic DNA from an organism of interest;

(3) mixing the primer according to step (1) with the genomic DNA according to step (2) under conditions appropriate to facilitate identification and isolation of a DNA fragment comprising a gene encoding an EGIII like cellulase.

On page 5, please replace the paragraph starting on line 19 with the following:

Fig. 3 illustrates a comparison (SEQ ID NO: 63) of a 102 residue peptide taken from the sequence of EGIII (SEQ ID NO: 43) with a corresponding peptide from *Fusarium equiseti [FUSEQIN]* (SEQ ID NO: 44); *Gliocladium roseum [GLIOIN]* (SEQ ID NO: 45); *Acremonium brachypenium [ACRHYPO]*(hypothetical protein sequence without intron) (SEQ ID NO: 46); *Aspergillus kawachii [ASPKAWA1]* (SEQ ID NO: 47); *Aspergillus aculeatus [ASPACU1]* (SEQ ID NO: 48); *Humicola insolens [HUMIN]* (SEQ ID NO: 49); *Actinomycete (streptomyces) sp. 11AG8 [11AG8IN]* (SEQ ID NO: 50); *Erwinia carotovara [ERWCARIN]* (SEQ ID NO: 51); *Gliocladium roseum [GLIO314]* (SEQ ID NO: 52); *Gliocladium roseum [GLIOHYP]*(hypothetical protein sequence without intron) (SEQ ID NO: 53); *Humicola grisea [HGRIS]* (SEQ ID NO: 54); *Rhodothermus marinus [RHMARIN]* (SEQ ID NO: 55); *Streptomyces lividans [SLIVINS]* (SEQ ID NO: 56); *Penicillium notatum [PENNOT]* (SEQ ID NO: 57); *Phanerochaete chrysosporium [PHANHYPO]*(hypothetical protein sequence without intron) (SEQ ID

NO: 58); *Emericella desertoru [EMDESHYP]*(hypothetical protein sequence without intron) (SEQ ID NO: 60); *Chaetomium brasillience* and *[CHBRAS]* (SEQ ID NO: 62); *Myceliopthora thermophila [MYCINS]* (SEQ ID NO: 61)(only 27 amino acids).

On page 8, please replace the paragraph starting on line 22 with the following:

Another embodiment comprising EGIII like enzymes according to the invention may be obtained according to the following methods. DNA primers are constructed which encode an amino acid sequence selected from the group consisting of one or more of:

(a) Asn-Asn-(Leu/Phe/Lys/Ile)-Trp-Gly (SEQ ID NO: 1)

(b) Glu-(Leu/Phe/Ile)-Met-Ile-Trp (SEQ ID NO: 2)

(c) Gly-Thr-Glu-Pro-Phe-Thr (SEQ ID NO: 2);

(d) (Ser/Tyr/Cys/Trp/Thr/Asn/Lys/Arg)-(Val/Pro)-(Lys/Ala)-(Ser/Ala)-(Tyr/Phe) (SEQ ID NO: 42); and

(e) Lys-Asn-Phe-Phe-Asn-Tyr (SEQ ID NO: 5).

and used to obtain DNA, and genes, encoding enzymes having cellulolytic activity according to established methods.

On page 31, please replace the paragraph starting on line 22 with the following:

The following DNA primers were constructed for use in amplification of EGIII like genes from the libraries constructed from the various microorganisms. All symbols used herein for protein and DNA sequences correspond to IUPAC IUB Biochemical Nomenclature Commission codes.

BOX1: primers coding for (N/Q)NLWG (SEQ ID NO: 64)

forward primer FRG001: AAY AAY YTN TGG GG (SEQ ID NO: 30)

forward primer FRG002: CAR AAY YTN TGG GG (SEQ ID NO: 31)

BOX1': primers coding for NNN(F/L/Y/I/L/N/K)WG (SEQ ID NO: 65)

forward primer FRG010: AAY AAY AAY HWI TGG GG (SEQ ID NO: 32)

BOX2: primers coding for ELMIW (SEQ ID NO: 66)

forward primer FRG003: GAR YTN ATG ATH TGG (SEQ ID NO: 33)

reversed primer FRG004: CCA DAT CAT NAR YTC (SEQ ID NO: 34)

BOX2': primers coding for YELMIW (SEQ ID NO: 67)

forward primer FRG011: TAY GAR YTI ATG ATH TGG (SEQ ID NO: 35)

reversed primer FRG012: CCA DAT CAT IAR YTC RTA (SEQ ID NO: 36)

BOX3: primers coding for GTE(P/C)FT (SEQ ID NO: 68)

reversed primer FRG005: GTR AAN GGY TCR GTR CC (SEQ ID NO: 37)

reversed primer FRG006: GTR AAN GGY TCR GTY CC (SEQ ID NO: 38)

reversed primer FRG007: GTR AAN GGY TCY GTR CC (SEQ ID NO: 39)

reversed primer FRG008: GTR AAN GGY TCY GTY CC (SEQ ID NO: 40)

reversed primer FRG009: GTR AAR CAY TCN GTN CC (SEQ ID NO: 41)

On page 32, please replace the paragraph starting on line 21 with the following:

The PCR strategy was as follows: forward primers for BOX1 and BOX1' were combined with reversed primers from BOX3 in a mixture with the desired genomic DNA sample and run on a gel to obtain fragments in the 400-1000 base pair range. The obtained fragments were then pooled and the pool split into two approximately equal portions. The first pool was combined with the forward primers from BOX1 and BOX1' along with the reversed primer from BOX2. The second pool was combined with the forward primer from BOX2 along with the reversed primers from BOX3. Fragments having the approximate size relative to an EGIII like cellulase considering the location of the primers within the gene, in this case corresponding to those between 250-500 base pairs, were isolated and sequenced. Partial sequences for EGIII like cellulase genes are provided in Fig. 3 (SEQ ID NOs: 43-62).

On page 32, please replace the paragraph starting on line 31 with the following:

The isolated and partially sequenced DNA and the corresponding amino acid sequences (of approximately 100 residues) were analyzed to determine their relationship to EGIII. The results of this sequence alignment are shown in Fig. 3 (SEQ ID NOs: 43-62). As shown in Fig. 3, significant sequence homology exists between the peptides encoded by the obtained DNA fragments (SEQ ID NOs: 44-62) and corresponding peptide sequences from EGIII (SEQ ID NO: 43). Due to this homology, it was concluded by Applicants that the nature of the numerous conserved residues identify the fragment as corresponding to a gene encoding a cellulase. Moreover, the high homology and strong conservation of residues corresponding to peptides (a), (b), (c) and/or (d), as in EGIII, identify the genes as coding an EGIII like enzyme from each of the organisms. Fig. 4 illustrates the percent similarity of the protein fragments sequenced.

On page 33, please replace the paragraph starting on line 6 with the following:

From the sequenced fragments, it was possible to use the RAGE technique (rapid amplification of genomic ends) to rapidly obtain the sequence of the full length gene. Full length genes were obtained and are provided with several additional EGIII-like cellulase sequences in Fig. 6 (SEQ ID NOs: 8-29). As shown in Fig. 6, full length genes isolated from *Hypocrea schweinitzii* (SEQ ID NO: 9), *Aspergillus aculeatus* (SEQ ID NO: 10), *Aspergillus kawachii (1)* (SEQ ID NO: 11), *Aspergillus kawachii (2)* (SEQ ID NO: 12), *Aspergillus oryzae* (SEQ ID NO: 13), *Humicola grisea* (SEQ ID NO: 14), *Humicola insolens* (SEQ ID NO: 15), *Chaetomium brasilliense* (SEQ ID NO: 16), *Fusarium equiseti* (SEQ ID NO: 17), *Fusarium javanicum (1)* (SEQ ID NO: 18), *Fusarium javanicum (2)* (SEQ ID NO: 19), *Gliocladium roseum (1)* (SEQ ID NO: 20), *Gliocladium roseum (2)* (SEQ ID NO: 21), *Gliocladium roseum (3)* (SEQ ID NO: 22), *Gliogladium roseum (4)* (SEQ ID NO: 23), *Memnoniella echinata* (SEQ ID NO: 24), *Actinomycete 11AG8* (SEQ ID NO: 26), *Streptomyces lividans CelB* (SEQ ID NO: 27), *Rhodothermus marinus* (SEQ ID NO: 28), *Emericella desertoru* (SEQ ID NO: 25), and *Erwinia carotovara* (SEQ ID NO: 29) all comprise significant homology EGIII from *Trichoderma reesei*.

Please replace the abstract with the following:

The present invention relates to novel enzymes which share certain conserved sequences with EGIII from *Trichoderma reesei*. These EG III like cellulases comprise an amino acid sequence comprising therein an amino acid string selected from the group consisting of:

(a) Asn-Asn-(Leu/Phe/Lys/Ile)-Trp-Gly (SEQ ID NO: 1)

(b) Glu-(Leu/Phe/Ile)-Met-Ile-Trp (SEQ ID NO: 2)

(c) Gly-Thr-Glu-Pro-Phe-Thr (SEQ ID NO: 3);

(d) (Ser/Tyr/Cys/Trp/Thr/Asn/Lys/Arg)-(Val/Pro)-(Lys/Ala)-(Ser/Ala)-(Tyr/Phe) (SEQ ID NO: 42);

(e) Lys-Asn-Phe-Phe-Asn-Tyr (SEQ ID NO: 5).

In the claims:

1. (Amended) An enzyme comprising cellulolytic activity comprising an amino acid sequence comprising an amino acid string selected from the group consisting of one or more of the following:

(a) Asn-Asn-(Leu/Phe/Lys/Ile)-Trp-Gly (SEQ ID NO: 1)

(b) Glu-(Leu/Phe/Ile)-Met-Ile-Trp (SEQ ID NO: 2)

(c) Gly-Thr-Glu-Pro-Phe-Thr; (SEQ ID NO: 3)

(d) (Ser/Tyr/Cys/Trp/Thr/Asn/Lys/Arg)-(Val/Pro)-(Lys/Ala)-(Ser/Ala)-(Tyr/Phe); (SEQ ID NO: 42) and

(e) Lys-Asn-Phe-Phe-Asn-Tyr (SEQ ID NO: 5)

or a derivative of said enzyme.

18. (Amended) A method for obtaining a gene encoding an EGIII like cellulase comprising the steps of

(a) preparing genomic DNA from an organism of interest;

(b) preparing a DNA primer encoding an amino acid string selected from the group consisting of one or more of the following:

(a) Asn-Asn-(Leu/Phe/Lys/Ile)-Trp-Gly (SEQ ID NO: 1)

(b) Glu-(Leu/Phe/Ile)-Met-Ile-Trp (SEQ ID NO: 2)

(c) Gly-Thr-Glu-Pro-Phe-Thr (SEQ ID NO: 2);

(d) (Ser/Tyr/Cys/Trp/Thr/Asn/Lys/Arg)-(Val/Pro)-(Lys/Ala)-(Ser/Ala)-(Tyr/Phe) (SEQ ID NO: 42); and

(e) Lys-Asn-Phe-Phe-Asn-Tyr (SEQ ID NO: 5).

(c) mixing said genomic DNA from step (a) and said DNA primer from step (b) under conditions suitable for the identification of all or part of a gene fragment in said genomic DNA corresponding to said DNA primer; and

(d) isolating said all or part of said gene corresponding to said fragment from said genomic DNA.